Supporting Information

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SI Text

Yeast Strains and Media. Strains used in this study are listed in Table S4. BY4741 (MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*) was used as the parental strain (1). The TAP and HTP carboxyl-tagged strains were generated by PCR as described (2). Strains were grown in 1% yeast extract, 2% peptone, 2% dextrose at 30 °C.

Oligonucleotides. Oligonucleotides used in this study are listed in Table S5.

DNA Manipulations. To construct plasmids containing the HTP tag sequence, PCR was performed with pBS1539 as template (2) with oligonucleotides that amplified a DNA fragment encoding 6 histidines (His_6), a spacer region, the TEV protease cleavage site, and 2 Protein A tags. The PCR product was cloned into pBS1539 using BamHI and HinDIII restriction sites, yielding pSG100. The GST-TEV protease expression construct (pGEX4T-TEV) was generated by ligating a BamHI–XhoI-digested TEV PCR product into pGEX4T-1 digested with the same restriction enzymes.

Extract Preparation and Affinity Purifications. For a single purification, 1 L of yeast culture was grown to an OD_{600} ≈0.5, and cells were harvested by centrifugation. For the in vivo cross-linking experiments, cells were resuspended in 1V/w of PBS and UV-irradiated (1.6 J/cm^2) in Petri dishes in a Stratalinker 1800 (Stratagene). Cells were pelleted and snap-frozen in liquid nitrogen. Cell pellets (≈1–1.5 g) were resuspended in 1.5 mL of lysis buffer [50 mM Tris·HCl (pH 7.8), 1.5 mM $MgCl_2$, 150 mM NaCl, 0.1% Nonidet P-40, and 5 mM β-mercaptoethanol], and extracts were prepared by vortexing the cell suspension with 3 mL of Zirkonia beads (0.5 mm; Thistle Scientific) 5 times for 1 min, with a 1-min incubation on ice in between each step. Three milliliters of lysis buffer was added and extracts were clarified by centrifugation (20 min at 4,500 × *g* and 20 min at 20,000 × *g* at 4 °C). Extracts were incubated with 250 μL of equilibrated IgG Sepharose beads (GE Healthcare) for 2 h at 4 °C. After 3 washes with 10 mL of lysis buffer, the beads were resuspended in 600 μL of lysis buffer containing 10 μg of home-made GST-TEV protease and incubated for 2 h at 18 °C. The suspension was subsequently transferred to micro bio spin columns (BioRad), and TEV eluates were collected by centrifugation.

Guanidine-HCl (0.4 g) was dissolved in 500 μL of TEV eluates (6 M final), and NaCl and imidazole were added to final concentrations of 300 and 10 mM, respectively. Samples were added to 50 μL of preequilibrated nickel-NTA beads (Qiagen) and agitated for 3 h or overnight at 4 °C. Beads were washed twice with 750 μL of wash buffer I [6 M guanidine·HCl, 50 mM Tris·HCl (pH 7.8), 300 mM NaCl, 0.1% Nonidet P-40, 10 mM imidazole, and 5 mM β-mercaptoethanol] and twice with 750 μL of wash buffer II [50 mM Tris·HCl (pH 7.8), 50 mM NaCl, 0.1% Nonidet P-40, 10 mM imidazole, and 5 mM β-mercaptoethanol]. UV cross-linked RNP complexes were eluted in 400 μL of elution buffer (wash buffer II but with 150 mM Imidazole).

Cloning of Cross-Linked RNA Fragments. Nucleotide sequences of linker, reverse transcription and PCR oligonucleotides are listed in Table S5. Except for the T4 RNA ligase (NEB), none of the enzymes used contained a His_6 tag.

Five hundred microliters of TEV eluate was incubated with 1 unit of an RNase A and T1 mixture (RNase-IT; Stratagene) between 5 and 20 min at 37 °C. Subsequently, 0.4 g of guanidine-HCl was added to the TEV eluates, and NaCl and imidazole were added to final concentrations of 300 and 10 mM, respectively. Samples were incubated overnight with 50 μL of nickel agarose beads (Qiagen). Beads were washed twice with 750 μL of wash buffer I and 3 times with 750 μL of 1× PNK buffer [50 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, 0.5% Nonidet P-40, 10 mM β-mercaptoethanol]. After transferring to microbio spin columns (BioRad), the beads were resuspended in 80 μL of prewarmed 1× PNK buffer containing 8 units of TSAP alkaline phosphatase (Promega) and 80 units of RNasin (Promega) and incubated at 37 °C for 30 min. Beads were washed with 400 μL of wash buffer I to inactivate the enzyme and washed 3 times with 400 μL of 1× PNK buffer. Beads were subsequently resuspended in 80 μL of 3′ linker ligation mix [1× PNK buffer containing 40 units of RNasin (Promega), 40 units of T4 RNA ligase (NEB), and 80 pmol of 3′ linker miRCat-33 or 3′ Solexa linker (Integrated DNA Technologies)] and incubated at 25 °C for 6 h. After washing the beads once with 400 μL of wash buffer I and 3 times with 400 μL of 1× PNK, beads were resuspended in 80 μL of labeling mix [40 μCi ^{32}P-γATP, 1× PNK buffer, 40 units of RNasin (Promega) and 20 units T4 polynucleotide kinase (PNK; purified from phage infected bacteria, Sigma)] and incubated for 40 min at 37 °C. Cold ATP was added to 1 mM and the reaction was allowed to continue for 20 min at 37 °C. Beads were washed once with 400 μL of wash buffer I and 3 times with 400 μL of 1× PNK. The beads were subsequently resuspended in 80 μL of 5′ linker ligation mix [1× PNK buffer containing 80 units of RNasin, 40 units of T4 RNA ligase, 100 pmol RL5 or Solexa 5′ linker, and 1 mM ATP] and incubated at 16 °C overnight. Radiolabeled RNPs were eluted in 400 μL of elution buffer [50 mM Tris (pH 7.8), 50 mM NaCl, 150 mM Imidazole, 0.1% Nonidet P-40, and 5 mM β-mercaptoethanol], TCA-precipitated, and resolved on 4–12% Bis-Tris NuPAGE gels (Invitrogen). RNPs were transferred to a nitrocellulose membrane and detected by autoradiography. Bands corresponding to the size of the protein of interest, including a region ≈0.5 cm above the band, were cut from the nitrocellulose membrane. Radiolabeled RNA was extracted by incubating the membrane slices with 100 μg of proteinase K in wash buffer II containing 1% SDS and 5 mM EDTA. RNA was subsequently phenol-chloroform-extracted and ethanol-precipitated. Reverse transcription with SuperScript III was performed as per manufacturer's procedures (Invitrogen). One microliter of cDNA was used in a 50-μL PCR containing 2.5 units of LA Taq Polymerase (Takara Bio), 1× LA TaqPCR buffer, 0.2 μM of each PCR primer (Table S5), and 0.25 mM dNTPs. PCR program was: 95 °C, 2 min, 98 °C, 20 s, 52 °C, 20 s, 68 °C, 20 s, 20–30 cycles, then 72 °C for 5 min. PCR products were resolved on 3% Methaphor agarose gels (Lonza), and PCR fragments with insert sizes from ≈10 to 50 bp were excised from the gel, purified, and cloned into the pCR4-TOPO vector per manufacturer's instructions and transformed into DH5α *E. coli*.

Sequencing. *E. coli* strains were grown in 96-well plates in LB medium supplemented with 50 $\mu g \cdot mL^{-1}$ ampicillin at 37 °C overnight. Two microliters of culture was used in a standard PCR using T7 and SP6 oligonucleotides to amplify the insert. To clean up the PCRs, 1 unit of shrimp alkaline phosphatase (GE Healthcare) and 0.075 units of Exonuclease I (NEB) was added and reactions were incubated for 40 min at 37 °C. After inactivating the enzymes at 80 °C, 2 μL of PCR product was sequenced in a BigDye Terminator Cycle Sequencing ready reaction (ABI

Prism) with a T7 primer on Applied Biosystems 9800 fast thermal cyclers, according to the manufacturer's procedures. Sequencing reactions were subsequently processed on ABI3730 capillary sequencing instruments (ABI Prism).

For the high-throughput sequencing analysis, cross-linked RNAs were ligated to the Solexa linkers and amplified by RT/PCR using oligonucleotides listed in Table S5. Illumina Solexa sequencing (single end 50-bp reads) was performed according to the manufacturer's procedure (Illumina).

Sequence Analysis. Sanger sequencing results were first analyzed with Blast (http://blast.ncbi.nlm.nih.gov) to locate linkers. Sequence fragments (inserts) found between pairs of linkers were extracted and analyzed with a second round of blast for homology with yeast coding and noncoding RNAs. To map insert sequences to U3 or ribosomal RNA, we typically used a Blast database consisting of a single sequence of interest, word size, 8; expectation value, 0.1; other parameters were set to default. Mapping inserts against a genomic blast database or changing blast parameters did not qualitatively affect the results. We then computed, for each position along the sequence of interest, the number of inserts covering that position.

Illumina Solexa sequences were mapped to the yeast genome and a noncoding RNA database (available from www.yeastgenome.org) using novoalign version 1.5 (www.novocraft.com). Novoalign finds global optimum alignments using the Needleman-Wunsch algorithm and allows mapping of sequences containing several substitutions or indels with respect to the reference sequence. Sequences were mapped using default settings, except that the strategy for reporting repeats (−r) was set to random, which randomly locates sequences that have multiple alignment locations, thereby evenly distributing the hits among all locations. Before mapping, the 3′ linker sequence was removed. We then calculated the number of reads, base substitutions, and base deletions mapped to each position in the reference sequences. We used base-quality filters (quality $>=$ 40 in the Nop1 and Nop56 experiments, quality $>=$ 5 in the Nop58 experiment) for mapping of substitutions and deletions. We visualized the distribution of reads along the yeast genome using Integrated Genome Browser (IGB; www.affymetrix.com/partners_programs/programs/developer/tools/download_igb.affx) and the distribution along individual genes using gnuplot (www.gnuplot.info/). IGB sgr files that depict the hits on the entire yeast genome and a set of awk and perl scripts used for sequence analysis are available on request.

Statistical Analysis. To analyze the relative enrichment of protein–RNA crosslinks in the vicinity of methylation sites, we downloaded a list of 63 known snoRNA-guided rRNA 2′-O-methylation sites from the University of Massachusetts Amherst Yeast snoRNA Database (http://people.biochem.umass.edu/sfournier/fournierlab/snornadb/main.php). For each position in the rDNA we calculated the number of hits, i.e., the number of reads mapped to that position. We normalized the numbers of hits by dividing these numbers by the number of all sequences mapped to rDNA and subtracting the normalized numbers of reads measured in the control experiment (negative values were set to 0). The proportion of hits in the vicinity of a methylation site was expressed as N_vicinity/N_total, where N_vicinity is the sum of normalized numbers of hits for all positions within 20 bp from a methylation site, and N_total is the sum of normalized numbers of hits for all positions in the rDNA. To estimate the expected proportion of hits in the vicinity of a methylation site under the null hypothesis (reads distributed randomly on the rDNA), we calculated the proportion of nucleotides in the rDNA that were located within 20 bp from a methylation site.

Northern Blot Analysis. To purify UV cross-linked RNAs, SDS and EDTA were added to samples to 1% and 5 mM, respectively and incubated with 100 μg of proteinase K (Roche) for 2 h at 55 °C, followed by phenol-chloroform extraction and ethanol precipitation of the RNA. For Northern blot analysis, RNA was resolved on 6% PAA-8 M urea gels and transferred to Hybond N^+ (GE Healthcare). Northern blot hybridization and generation of randomly-primed U3 snoRNA probes was done as described (3). Primer extension with radiolabeled oligonucleotides was performed by using SuperScript III reverse transcriptase per the manufacturer's procedures (Invitrogen). The cDNAs were phenol chloroform-extracted, ethanol-precipitated, resolved on 6% PAA-8 M urea sequencing gels, and detected by autoradiography.

Western Blot Analysis. Nickel eluates and 5% of UV-irradiated TEV eluates were incubated with 10 μg of RNase A for 30 min at 37 °C. Proteins were precipitated with 20% TCA, resolved on 4–12% Bis-Tris NuPAGE gradient gels (Invitrogen), and transferred to a nitrocellulose membrane. TAP- and HTP-tagged proteins were detected by using polyclonal rabbit anti-TAP antibodies (Open Biosystems).

Primer Extension Analysis. Primer extension analysis on the U3 snoRNA was performed by the manufacturer's procedures (SuperScript III; Invitrogen). All reactions were performed at 50 °C using 1 pmol of labeled primer. RNAs were phenol-chloroform-extracted and resolved on 6% PAA-UREA sequencing gels and detected by autoradiography.

Purification of GST-TEV Protease. BL21 DE3 cells (Invitrogen) transformed with pGEX4T-TEV were grown in 2 L of LB medium supplemented with 50 μg/mL ampicillin at 37 °C to an OD_{600} of 0.5 and the culture was then cooled to 18 °C. Protein expression was induced with 100 μM IPTG and the culture was incubated at 18 °C overnight. Cells were harvested by centrifugation and resuspended in 1 V/g cells of breaking buffer [50 mM Tris·HCl (pH 7.9), 150 mM NaCl, 10% sucrose, 0.1% Nonidet P-40, 10 mM β-mercaptoethanol]. Five milliliters of Zirkonia beads (0.5 mm; Thistle Scientific) was added, and the suspension was vortexed 3 times for 1 min, with a 1-min incubation on ice in between each step. Clarified extracts were fractionated on a 30-mL SP-Sepharose column (GE Healthcare) essentially as described (4). Briefly, after passing the *E. coli* extract through the SP-Sepharose column, the column was washed with 3 volumes of T50 [50 mM Tris·HCl (pH 7.8), 50 mM NaCl, 0.1% Nonidet P-40, 10 mM β-mercaptoethanol] and proteins were eluted with a linear salt gradient (50–600 mM NaCl). Fractions containing GST-TEV were pooled and the protease was further enriched by glutathione Sepharose affinity chromatography as per manufacturer's procedures (GE Healthcare). GST-TEV protease was dialyzed in TEV storage buffer [50% glycerol, 50 mM Tris·HCl (pH 7.8), 0.1% Nonidet P-40, 1 mM EDTA, 10 mM β-mercaptoethanol] overnight at 4 °C and aliquots were stored at −80 °C.

Data. Because the U3 snoRNA is relatively small (333 nt), we reasoned we could map the protein binding sites on the U3 snoRNA by primer extension by using only a few oligonucleotides (5, 6). We first catalogued all of the UV-induced primer extension stops in the U3 snoRNA. UV-irradiated TEV eluates were proteinase K-treated and extracted RNA was analyzed by primer extension using several radiolabeled oligonucleotides (Table S5) to cover the entire U3 snoRNA sequence. The cDNAs were resolved on 6% PAA-urea gels and detected by autoradiography (Fig. S1*a*).

Comparison of irradiated U3, from phenol-chloroform-treated TEV eluates, with irradiated and untreated U3 snoRNP, identified a large number of primer extension stops present specifically in U3 snoRNA recovered from UV-irradiated U3

snoRNP particles (Fig. S1*a*). The deproteinized, irradiated U3 closely resembled the untreated sample, whereas numerous additional stops were detected in the irradiated RNP sample. This indicates that the RNP is much more susceptible to cross-linking than is the naked RNA, possibly because of a more densely-packed structure. UV-B light irradiation can potentially introduce RNA strand breaks, but this was not detected at these levels of UV exposure in the deproteinized U3.

An overview of all primer extension stops specifically identified in the UV-irradiated U3 snoRNP is shown in Fig. S1*b*. Most of the stops were found at uridine residues and are located in regions proposed to be single-stranded. Interestingly, a number of primer extension stops were located at or near conserved and functionally important regions. Three primer extension stops were identified in the conserved stem II (Fig. S1*b*; C259-U261), of which C259 has been shown to be important for efficient association of Rrp9 to the U3 snoRNA in yeast (7). In addition, 3 stops were located in a region that base-pairs with pre-rRNA (8) and 2 stops were found in the conserved box A in helix 1a (Fig. S1*b*; U22-U23). Finally, 2 stops were found near box C′ (Fig. S1*b*; U77-G78). However, most primer extension stops were equally detected in RNA recovered in association with Rrp9 and other proteins tested. The low apparent efficiency of protein-RNA cross-linking makes it unlikely that single RNA molecules were cross-linked to multiple proteins. This indicates that these are RNA-RNA cross-links rather than protein–RNA cross-links (Fig. S1*c*). In contrast, 2 primer extension stops, located between helix 4 and helix 2 in the U3 snoRNA (U198-G199), were reproducibly enriched in Rrp9 nickel eluates. This indicated that U198-G199 represent sites of Rrp9-U3 interaction.

1. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.
2. Rigaut G, et al. (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* 17:1030–1032.
3. Houseley J, Kotovic K, El Hage A, Tollervey D (2007) Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. *EMBO J* 26:4996–5006.
4. Granneman S, *et al.* (2006) The nucleolar protein Esf2 interacts directly with the DExD/H box RNA helicase, Dbp8, to stimulate ATP hydrolysis. *Nucleic Acids Res* 34:3189–3199.
5. Urlaub H, Hartmuth K, Kostka S, Grelle G, Luhrmann R (2000) A general approach for identification of RNA–protein cross-linking sites within native human spliceosomal small nuclear ribonucleoproteins (snRNPs). Analysis of RNA–protein contacts in native U1 and U4/U6.U5 snRNPs. *J Biol Chem* 275:41458–41468.
6. Urlaub H, Hartmuth K, Luhrmann R (2002) A two-tracked approach to analyze RNA–protein cross-linking sites in native, nonlabeled small nuclear ribonucleoprotein particles. *Methods* 26:170–181.
7. Clery A, Senty-Segault V, Leclerc F, Raue HA, Branlant C (2007) Analysis of sequence and structural features that identify the B/C motif of U3 small nucleolar RNA as the recognition site for the Snu13p–Rrp9p protein pair. *Mol Cell Biol* 27:1191–1206.
8. Beltrame M, Tollervey D (1992) Identification and functional analysis of two U3 binding sites on yeast pre-ribosomal RNA. *EMBO J* 11:1531–1542.

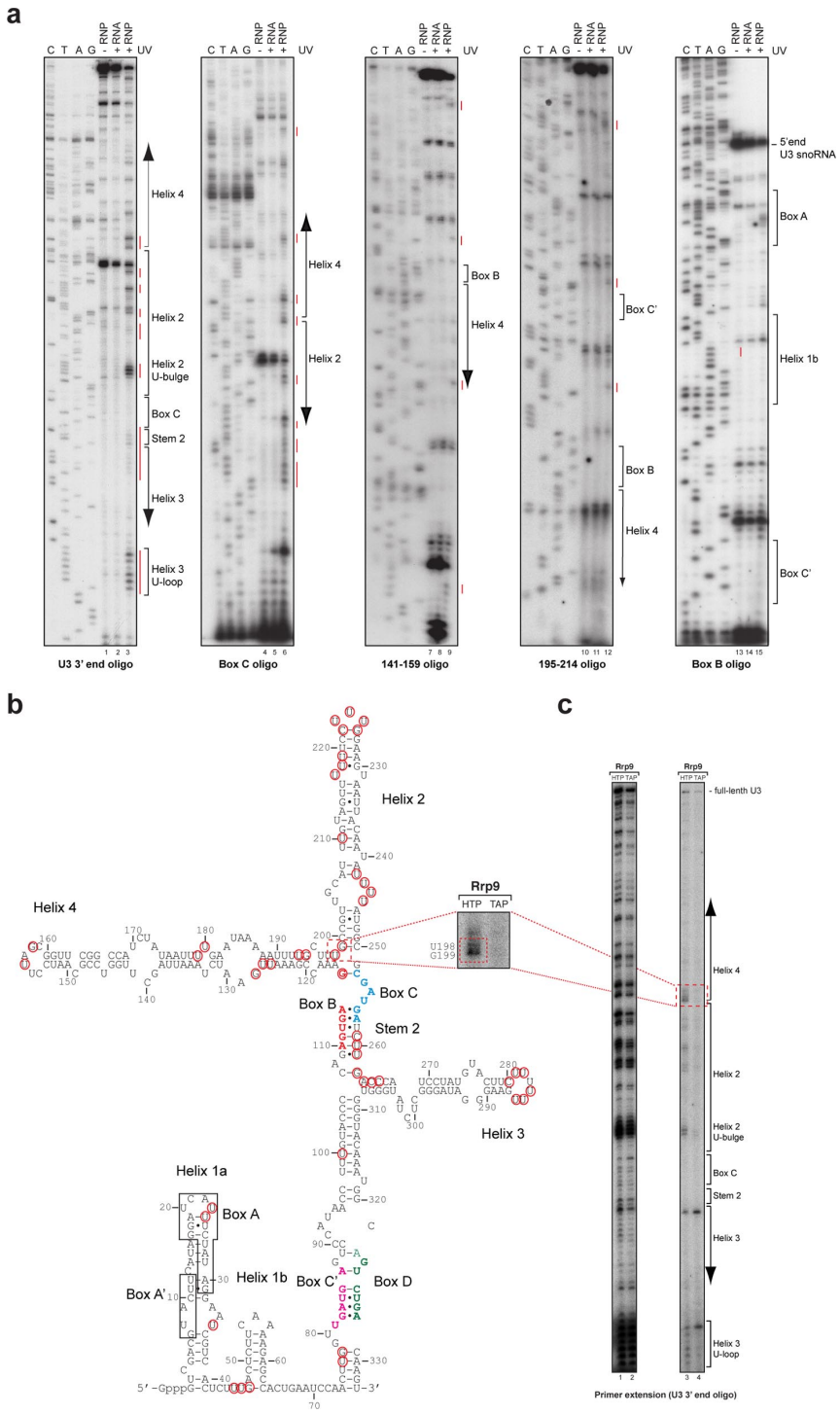

Fig. S1. Primer extension analysis identifies potential Rrp9 RNA binding sites at U198-G199 in U3. (*a*) Primer extension analysis of U3 snoRNA extracted from untreated U3 snoRNP (RNP − UV), deproteinized, and UV-cross-linked U3 snoRNA (RNA + UV) and UV-cross-linked U3 snoRNP particles (RNP + UV). C, T, A, and G indicate dideoxynucleotides used for sequencing reactions. The oligonucleotides used for primer extension are listed below each panel. Arrows and brackets indicate sequences of helices 1–4, stem 2, and other functionally-important sequence elements (box A, A′, B, C, C′, and D) of the U3 snoRNA. Red bars on the right side of each panel indicate primer extension stops in the U3 snoRNA that are only found in UV-irradiated U3 snoRNP. (*b*) Schematic representation of primer extension stops found specifically in UV-irradiated U3 snoRNP. Open red circles indicate primer extension stops present only in U3 RNA of UV cross-linked U3 snoRNP. Boxed nucleotides indicate the conserved boxes A and A′. Colored nucleotides indicate conserved B (red), C (blue), C′ (pink), and D (green). (*c*) Rrp9 may bind the U3 snoRNA near U198 and G199 of the U3 snoRNA. Primer extensions were performed on in vitro cross-linked U3 snoRNA extracted from nickel-purified His-6-tagged Rrp9. The rrp9-TAP strain was used as a negative control. Two percent input indicates primer extension reactions performed on RNA extracted from 2% of the TEV eluates. Arrows and brackets indicate sequences of helices and bulges, conserved stem 2, and box C. Red bars on the right side of each panel indicate primer extension stops in the U3 snoRNA only found in UV-irradiated U3 snoRNP.

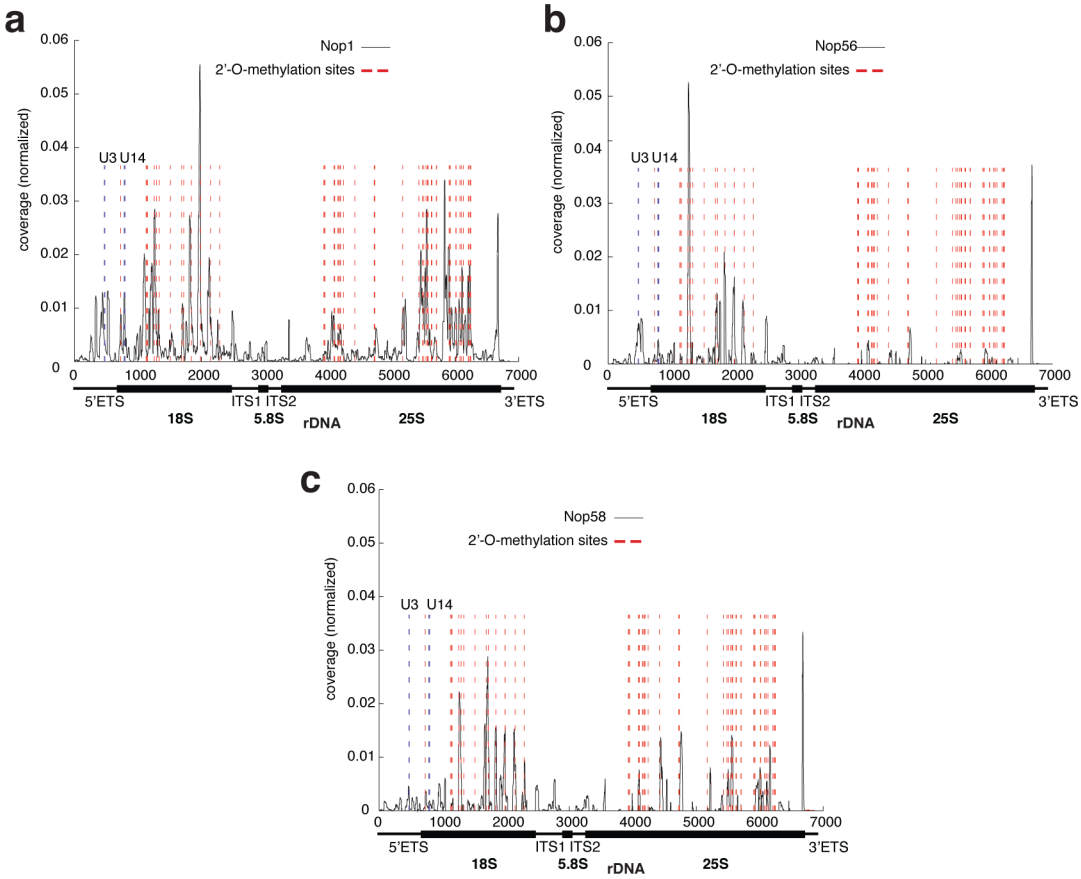

Fig. S2. Cross-linking of common box C/D snoRNP proteins to pre-rRNA normalized to the control data. The raw data are presented in Fig. S3. The graphs display the percentage of sequences in the Nop1 (*a*), Nop56 (*b*), and Nop58 (*c*) deep sequencing data that mapped to the rDNA (*x* axis). We calculated the percentage of hits for each nucleotide in the rDNA by dividing the total number of hits for a particular nucleotide by the total number of rDNA hits. Using these numbers, we then normalized the Nop1, Nop56, and Nop58 data by subtracting the numbers from the control data. Any negative values were set to 0. The red dotted lines indicate the methylation sites. The blue dotted lines indicate the U3 and U14 snoRNA base-pairing sites, 2 RNAs involved in pre-rRNA processing reactions. The location of ETSs (5′ETS and 3′ETS), mature rRNA sequences (18S, 5.8S, 25S), and internal transcribed spacers (ITS1 and ITS2) on the *x* axis rDNA is depicted below the *x* axis.

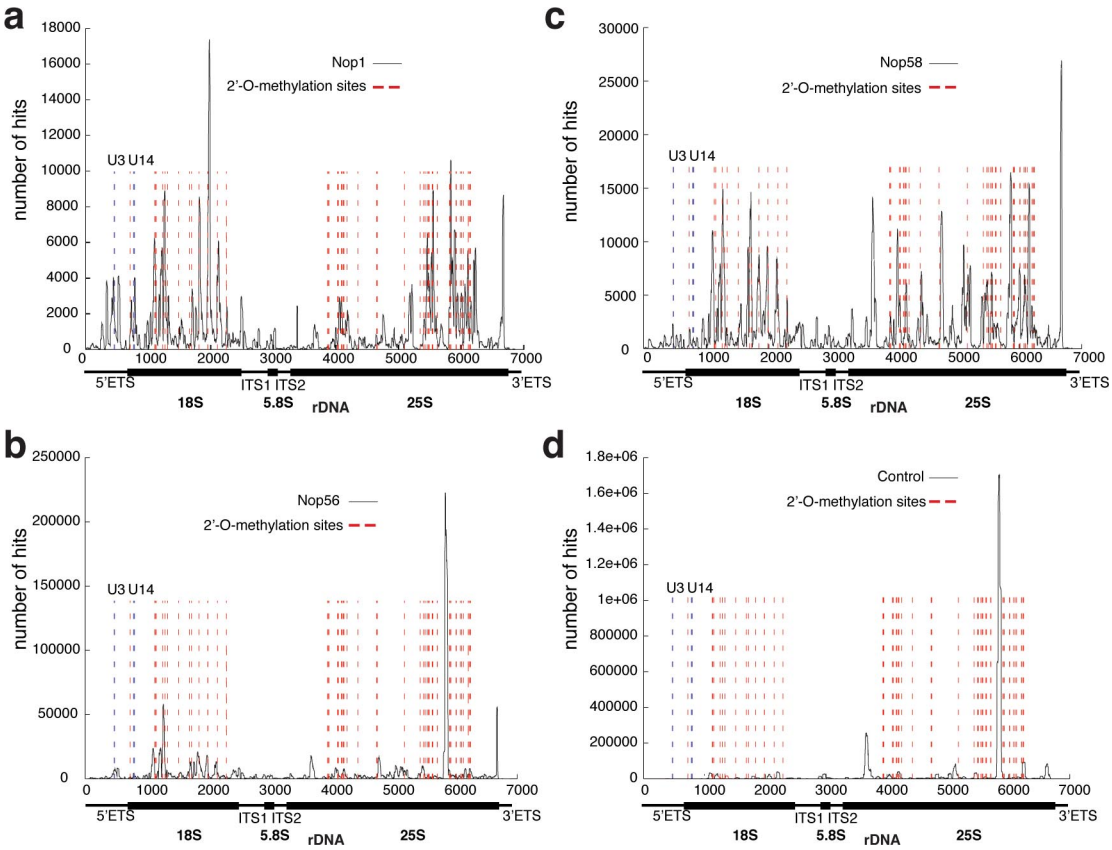

Fig. S3. The core box C/D snoRNP proteins cross-link to pre-rRNA preferentially at 2′-O-methylation sites. In these graphs the raw data are shown. The graphs display the number of sequences in the Nop1 (*a*), Nop56 (*b*), Nop58 (*c*), and control (*d*) deep sequencing data that mapped to the rDNA (*x* axis). The red dotted lines indicate the methylation sites. The blue dotted lines indicate the U3 and U14 base-pairing sites, 2 RNAs involved in pre-rRNA processing reactions. The location of ETSs (5′ETS and 3′ETS), mature rRNA sequences (18S, 5.8S, 25S), and internal transcribed spacers (ITS1 and ITS2) on the *x* axis rDNA is depicted below the *x* axis.

Table S1. Overview of the CRAC deep sequencing results

RNAs identified in mapped sequence reads	Tagged bait proteins			
	Nop1	Nop56	Nop58	Control
Total yeast genome				
snoRNA	88.55	79.20	71.00	0.67
C/D snoRNAs	87.58	78.78	70.00	0.53
H/ACA snoRNAs	0.94	0.41	1.00	0.14
U3 snoRNA	11.01	7.20	9.38	0.11
rRNA	8.83	16.30	22.00	83.34
tRNA	0.05	0.15	0.00	0.52
snRNA	0.46	0.06	0.00	0.29
CDS	3.21	6.86	6.00	42.16
Average mapped read length, nt	22.10	33.60	24.95	48.22
Total mapped sequences	2,526,260	5,087,959	1,555,336	4,362,938
Noncoding RNA database				
SnoRNA	90.22	81.36	74.30	0.79
C/D snoRNAs	89.07	80.77	71.87	0.79
H/ACA snoRNAs	1.15	0.59	2.43	0.00
U3 snoRNA	13.26	7.63	8.95	0.13
rRNA	9.21	18.21	25.08	98.47
tRNA	0.44	0.32	0.41	0.38
snRNA	0.06	0.05	0.03	0.33
Other	0.06	0.06	0.18	0.04
Average mapped read length, nt	22.06	32.60	25.00	48.20
Total mapped sequences	2,774,914	4,819,644	1,642,375	4,362,938

Four to six pmol of PCR product was used for Solexa sequencing to ensure that approximately an equal number of reads for each sample could be analyzed. Solexa sequences were mapped to both the entire yeast genome and a noncoding RNA database. Shown are the percentage of sequences from the CRACS data sets that were assigned to a specific class of noncoding RNAs, the averages length of sequences found between the linkers, and the total number of sequences that were mapped. CDS: coding sequence, which are all the coding sequences of protein coding genes. Other: other noncoding RNAs. The smaller size of the noncoding RNA database allows more sequences to be aligned, increasing the total number.

Table S2. Overview U3 snoRNA fragments identified by manual sequencing of cDNA clones aligned to the U3a sequence

Fragment	Sequence
Nop1_63	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_11	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_80	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_74	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAG*G*
Nop1_70	AATGGCAGT**CTGA**CAAGT
Nop1_71	AAATGGCAGT**CTGA**CAAGT
Nop1_40	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_44	CTATGGGTGGNTAC*G*AATGGCAGT**CTGA**CAAGT
Nop1_30	ATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_15	ACAAATGGCAGT**CTGA**CAAGT
Nop1_25	AAATGGCAGT**CTGA**CAAG
Nop1_30	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_05	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_33	TTTGAAGGGATAGGGCTATGGGTGGGTACAGATGGCAGT**CTGA**CAA
Nop1_A_02	CTATGGGTGGGTACATGGCAGT**CTGA**CAAGT
Nop1_69	GGCTCCATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_77	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop1_34	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAG*A*
Nop56_T_19	AGGGCTCTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop56_T_46	GCTCTATGGGTGGGTACAAATGGCAGTCTGACAAG
Nop56_F_02	CTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop56_D_01	AGGGCTCTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop56_E_05	GAAGGGATAGGGCTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop56_E_07	ATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop56_E_12	AGGGCTCTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop58_T_06	TGGGTGGGTACAAATGGCA*T*T**CTGA**CAAGT
Nop58_T_26	ATGGGTGGGTACAAATGGCA*T*T**CTGA**CAAGT
Nop58_T_38	ATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop58_T_27	ATGGGTGGGTACAAATGGT**CTGA**CAAGT
Nop58_H_09	ATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT
Nop58_G_05	TATGGGTGGGTACAAATGGCT**CTGA**CAAGT
Nop58_B_03	AGT**CTGA**CAAGT
U3a	285-TTGAAGGGATAGGGCTCTATGGGTGGGTACAAATGGCAGT**CTGA**CAAGT-333

The conserved box D is indicated in bold. Substitutions and deletions are indicated in italics.

Table S3. Enrichment of protein–rRNA cross-links in the proximity of known rRNA 2′-O-methylation sites

	18S*		25S†	
Site	Observed‡	Random§	Observed†	Random§
Nop1	59.95	32.38	65.30	26.80
Nop56	57.47	32.38	24.20	26.80
Nop58	59.21	32.38	37.87	26.80

*Reads mapped between positions 701 and 2516 in the rDNA.
†Reads mapped between positions 3267 and 6688 in the rDNA.
‡Number of nucleotide hits within 20 bp from a known 2′-O-methylation site, divided by the total number of nucleotide hits in the rDNA.
§Expected proportion of hits within 20 bp from a methylation site, if reads were distributed randomly on the rDNA.

Table S4. Yeast strains used in this study

Strain	Genotype	Ref.
BY4741	MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*	1
YSG487	As BY4741 but with *rrp9-HTP::K.l.URA3*	This study
YSG494	As BY4741 but with *rrp9-TAP::K.l.URA3*	This study
YSG555	As BY4741 but with *nop1-HTP::K.l.URA3*	This study
YSG556	As BY4741 but with *nop56-HTP::K.l.URA3*	This study
YSG558	As BY4741 but with *nop58-HTP::K.l.URA3*	This study

1. Brachman CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.

Table S5. Oligonucleotides used in this study

Name	Sequence	Ref.
Cloning linkers		
miRCat-33 3′ linker	5′-rAppTGGAATTCTCGGGTGCCAAGG/ddC/-3′	1
RL5	5′-*AGGGAGGACGAUGCGG*-3′	2
Solexa 3′ linker	5′-rAppTCGTATGCCGTCTTCTGCTTGT/ddC/-3′	This study
Solexa 5′ linker	5′-/InvddT/GTT*CAGAGUUCUACAGUCCGACGAUC*-3′	This study
Oligonucleotides for reverse transcription		
miRCat-33 RT	5′-CCTTGGCACCCGAGAATT-3′	This study
Solexa RT	5′-CAAGCAGAAGACGGCATAC-3′	This study
Oligonucleotides for PCR:		
DP5	5′-AGGGAGGACGATGCGG-3′	2
miRCat-33 Rev	5′-GCCTTGGCACCCGAGAATTCC-3′	This study
Solexa PCR Forw	5′-AATGATACTGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA-3′	This study
Solexa PCR Rev	5′-CAAGCAGAAGACGGCATACGA-3′	This study
Primer extension oligonucleotides		
U3 3′ end oligo	5′-ACTTGTCAGACTGCCAT-3′	This study
Box C oligo	5′-GGTCAAGATCATCGCGCCAT-3′	This study
U3 141–159 oligo	5′-GCTAAGGATTGCGGACCAA-3′	This study
U3 195–214 oligo	5′-CTACAAATGCAACGGCAAAG-3′	This study
Box B oligo	5′-GGTTTCTCACTCTGGGGTAC-3′	This study

RNA sequences are italicized.

1. Pak J, Fire A (2007) Distinct populations of primary and secondary effectors during RNAi in *C. elegans*. *Science* 315:241–244.
2. Ule J, et al. (2003) CLIP identifies Nova-regulated RNA networks in the brain. *Science* 302:1212–1215.